

# Rheology of two-dimensional F-actin networks associated with a lipid interface

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We report on the surface rheology of cross-linked F-actin networks associated with a lipid monolayer at the air-water interface of a Langmuir monolayer. The rheological measurements are made using a Couette cell. These data demonstrate that the network has a finite elastic modulus that grows as a function of the cross-linking concentration. We also note that under steady-state flow the system behaves as a power law fluid in which the effective viscosity decreases with imposed shear.

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## I. INTRODUCTION

The cytoskeleton of eukaryotic cells is a chemically heterogeneous assemblage of filamentous proteins, cross-linking agents, and molecular motors. This stiff filamentous network is mechanically linked to the plasma membrane and comprises the principal structural element of the cell. It confers mechanical stability and is the site of force generation and morphological control of the cell body. As such it is essential for such biologically important processes as intracellular trafficking, cell motility, and the measurement of applied stress.

In the past great progress has been made in elucidating the underlying physical properties of the cytoskeleton by building highly simplified *in vitro* model systems, which provide controlled laboratories for exploring the mechanics of chemically well-controlled systems extracted from the complex cytoskeleton. From this work has emerged a well-developed model of semiflexible F-actin networks and solutions. While much is currently known about the mechanics of semiflexible networks [1, 2, 3, 4], less is understood about the mechanical properties of this biopolymer network when mechanically associated with the fluid plasma membrane of the cell. One reason for this is that the coupling of a semiflexible network to the cell membrane is a distinct feature of cellular mechanics [5, 6, 7, 8] that has not been as well studied using simplified *in vitro* models. Previous work in this area has been done using indentation-based mechanical probes of F-actin coated lipid vesicles [9] and microrheology [10, 11].

In this work we present a new biomimetic model for this composite structure that we believe is more amenable to detailed mechanical study and the visualization of the strain field within it. We have developed a cross-linked F-actin network that is mechanically associated with a DPPC monolayer in a Langmuir trough by biotin/streptavidin bonds. This coupling approximates the more complex linkage between the physiological cytoskeleton and the plasma membrane effected via specialized protein complexes [12]. It is important to keep in mind that this is truly a minimal model of the composite membrane/cytoskeleton system. The full biological system includes significantly more complex linkages between

actin filaments and the actin-lipid connection. For example, there are multiple inter-filament cross-links, some of which introduce interesting elastic properties (such as Filamin linkers) [13, 14, 15]. Many physiologically relevant cross-linking proteins are labile generating transient bonds. The cross-linking scheme employed here, however, creates permanent cross-links on the time-scale of the experiments. We briefly speculate on the rheological signature of such labile cross-linkers in the summary of this work. Even though the choice of a simpler system of linkage molecules severely limits its chemical complexity, it is precisely this choice of system that allows for reproducible, quantitative measurements in a system that is more amenable to theoretical investigation [16, 17, 18].

We suspect that the rheological effect of the monolayer is indirect. In this work, the lipid monolayer is in a highly fluid phase, so that it has no zero-frequency elastic modulus. We estimate that, at the frequencies probed here, the viscous stresses in the lipid monolayer are 2 - 3 orders of magnitude less than the elastic stresses in the actin network. Therefore, we do not expect it to make any direct contribution to the measured mechanical properties of the complex. By creating a thin-layer of F-actin bound to the monolayer, however, we anticipate that we have likely generated an anisotropic network in which the long F-actin filaments are tangent to the lipid decorated surface. This anisotropic structure reflects the coarse geometry of the cell's dense cortical cytoskeleton, and we explore the mechanics of this highly anisotropic network. Additionally, the monolayer serves as a protective barrier for the actin, preventing denaturation at the air-water interface. The thin-film geometry has other benefits not fully exploited in this work: one can directly examine the resulting planar strain field under stress. This feature of the system will be more fully discussed elsewhere. Finally, one can consider the binding of the network to denser lipid monolayers whose elastic moduli can compete with those of the F-actin network. However, the impact of denser monolayers on the response of the system will be the subject of future work [19].

We rheologically probe this network using a newly developed Couette-style, surface rheometer, the details of which will be presented elsewhere [20]. In this first report

on the mechanics of the F-actin/monolayer system, we focus primarily on the steady-state shearing of the network. In this state we expect that the F-actin network flows due to continual rearrangements of cross-linked or sterically jammed collections of filaments. These filament networks must transiently interpenetrate each other and then disengage during the flow. Though this regime does not have direct biological applications, it is important for the complete material characterization of the system. It provides important baseline measurements on the bulk elastic and viscous properties that will be useful in future microrheological studies of the network.

Under steady-state shear, we find that the material is highly nonlinear in its rheological response behaving as a shear-thinning, power-law fluid. The power-law exponent is weakly dependent on the density of permanent cross-links in the system. We find no evidence for a yield stress, and the observed elastic modulus is weakly dependent on shear rate. Based on these data we believe that our network consists of permanently bonded patches of filaments that interpenetrate each other; these patches exchange nearest neighbors during the shear. As such the network may be characterized as having many metastable states separated by low energy barriers. It has been suggested that such system should obey a type of universal “soft glassy rheology.” We find that our rheological data in this steadily flowing state is indeed consistent with the predictions of this theory [21].

By examining the initiation and cessation of shear, we explore the time-dependent viscoelastic response of the material. We find that features of the linear response of the F-actin network can be accounted for using a generalized Maxwell model having two stress relaxation times. Our data suggest that the elastic modulus of the network grows weakly, but in a linear fashion with cross-linker density; it also plateaus at higher cross-linking densities suggesting that steric hinderances prevent the saturation of all available cross-linkers in the system.

In the remainder of this article, we first discuss briefly the experimental design of the apparatus, as well our methods for analyzing the data in section II. We then present our data and discuss its interpretation in section III, before concluding with a discussion of the implications of this work to future investigations in section IV.

## II. MATERIALS AND METHODS

### A. Preparation of actin-lipid composite material

We create the F-actin monolayer *in vitro* by polymerizing for one hour G-actin monomers along with the variable fraction of biotinylated actin monomers in a F-actin buffer (Tris HCl buffer pH=7.4, 0.2 mM ATP, 3 mM MgCl<sub>2</sub>, 0.2 mM CaCl<sub>2</sub>). In each preparation we fixed the concentration of the non-biotinylated actin to 2 mg/mL. We use the parameter R, which is the ratio of biotinylated actin monomers to G-actin monomers, to characterize the

cross linking density of our actin filament networks. We polymerize the actin using this standard procedure to ensure that actin filaments are formed. With this procedure, we expect filaments that average 5  $\mu\text{m}$  in length. Because our apparatus requires relatively large volumes of fluid, we diluted these actin solutions 100X and added Rhodamine phalloidin and streptavidin. The phalloidin stabilizes the F-actin filaments against depolymerization during the experiment [23] while the streptavidin cross-links the biotinylated monomers creating a permanent, stress-bearing semiflexible polymer network. Once diluted, we estimate that the mesh size of the F-actin network is 2.7 $\mu\text{m}$  [22]. We waited 30 minutes for the completion of the cross-linking reaction before transferring the protein solution into the Couette trough and adding a lipid solution (93% DPPC, 7% DPPE - Biotynl) at the surface. We then allowed the system to equilibrate for one hour in order for the F-actin network to diffuse to and irreversibly bind to the biotinylated monolayer [24]. We then performed the rheological measurements over a period of about two hours. During this period we observed no evidence of a significant change in rheological properties reflecting either continued cross-linking or network degradation. These measurements were made using a Couette surface viscometer designed specifically for this work.

The depth profile of actin-lipid composite materials formed at the air-water interface has been previously characterized by neutron reflectivity experiments [25]. These experiments show that a monolayer of close packed actin filaments will bind to a layer of streptavidin and thus irreversibly bind to the lipid monolayer. Therefore, we expect that we have created a low yield stress solid associated with the air-water interface due to a combination of biotin-streptavidin cross-links and steric interactions between filaments. In this set of rheological measurements, we examine primarily the steady-state flow properties of this network under time-independent shearing imposed at the boundaries. We will explore the small strain elastic behavior of this material in future work [19].

### B. Couette Surface Rheology with Torsion Pendulum

The Couette surface viscometer is a modified Couette rheometer (see [26] for a description of the main principles of operation) designed specifically to measure the viscosity of surface films. The details of the instrument will be described in a separate publication [20]; however, its main features are reviewed here. The apparatus consists of a teflon cylindrical container forming the outer barrier (of radius  $r_o = 3.75$  cm) of the sample and an teflon inner barrier (of radius  $r_i = 1.25$  cm) coupled to a torsion pendulum. A wire coil attached to the torsion pendulum is positioned within a second set of coils. An ac current in the second set of coils generates a high fre-

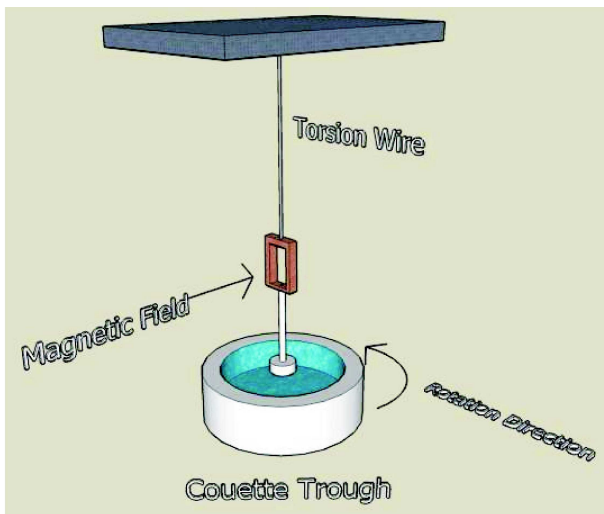


FIG. 1: A schematic diagram of the Couette trough used in the experiments. The F-actin and lipid monolayer completely fills the surface between the inner and outer teflon cylinders. The outer cylinder is rotated at constant angular velocity and angular displacement of the inner cylinder is monitored via the magnet rigidly coupled to torsion wire.

quency magnetic field that induces a voltage in the coil attached to the torsion pendulum. The angular position of the torsion pendulum is detected by the magnitude of the induced voltage in the attached coil. A schematic diagram of the experiment is shown in Fig. 1.

We used a lock-in amplifier to detect the small induced voltages at the same frequency but  $90^\circ$  out of phase with the reference signal powering the external magnetic field. The angular position as a function of induced voltage is calibrated using an angular translation stage. From the angular displacement we calculate the torque  $\mathcal{T}$  on the inner barrier using the torsion constant of the torsion pendulum, which we determined previously. The torque  $\mathcal{T}$  measured at the inner rotor (but constant throughout the system) of radius  $r_i$  can then be related to the shear stress there by geometry:

$$\sigma_{r\theta}|_{r=r_i} = \frac{\mathcal{T}}{2\pi r_i^2}, \quad (1)$$

where  $r$  and  $\theta$  are the radial and azimuthal coordinates respectively on the surface of the Langmuir monolayer. We suppress these indices hereafter. The raw data consists of torque versus time curves under constant rate of strain or after the cessation of applied strain. This data is converted into elastic modulus, viscosity, and relaxation time constants using well-defined procedures. However, two aspects of this rheometer are non-standard: it operates in a wide-gap geometry and the inner cylinder is free to move. Therefore, it is worth summarizing the impact of these features on the measurements of the elastic modulus and the viscosity. These will be discussed in the next section in the context of an example torque versus time curve.

### III. RESULTS

As previously discussed, we focus on steady-state, constant shear rate rheological studies to examine the changes in the rheology of the network as a function of cross-linker density. We also study the response of the system at the initiation and cessation of shear to examine the linear, time-dependent shear modulus. The studies of the initiation of flow allow for determination of a linear elastic modulus for the system, while examining the stress relaxation at the cessation of shear can be used to measure the stress relaxation time in the medium. We parameterize the linear, time-dependent stress response during the initiation and cessation of flow by introducing a simple Maxwell fluid model [27]. Examining the steady-state rheology, we observe a strong shear-rate dependence of our results that is well-described by a power-law fluid model [28]. Due to this nonlinear behavior, we do not expect that the simple Maxwell model will also capture the steady-state flow. By varying the density of cross-linkers, we study how the nonlinearity of the material under steady-state shear and how the elastic modulus of the network changes due to changes in the density of cross-linkers.

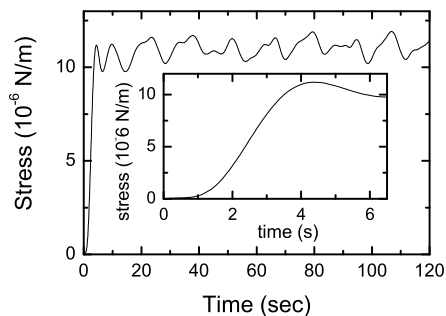


FIG. 2: Typical data set: The F-actin network has a cross linker density of  $R = 0.005$ . At  $t = 0$  the outer cylinder reaches a fixed angular velocity of  $0.4$  rad/sec (which corresponds to a shear rate of  $1.3 \text{ s}^{-1}$ ). The measured torque rapidly reaches a plateau and returns to zero when the outer cylinder is stopped (see Fig. 3). Insert shows a closeup of the initial rise.

All the data from the experiments performed using our Couette style apparatus share similar phenomenology. We show a typical example of the time evolution of the measured torque from the initiation of shear to steady-state in Fig. 2. In Fig. 3, we show stress decay at the cessation of shear and a linear fit to the essentially exponential decay of stress on a semilog plot (inset). At times before  $t = 0$ , the outer barrier is at rest; these data are used to zero the measurement of the torque. At  $t = 0$ , the outer barrier begins to rotate at a fixed angular velocity. Given the temporal resolution of the instrument we may consider this change in angular velocity to be effectively instantaneous. After a brief interval of stress overshoot, the system relaxes to a steady-state torque, which

can be interpreted in terms of the steady-state viscous response of the surface film. The observed fluctuations in the torque at steady-state system are larger in magnitude than those found in the stationary system. Therefore, the torque fluctuations in the steadily sheared state suggest the quasi-periodic build up and decay of internal stress-bearing structures associated with the monolayer. In this article, however, we focus on the time-averaged response of the material in the steady-state. Finally, once the rotation of the outer barrier ceases, the measured torque is observed to decay to zero in an exponential fashion – see Fig. 3. We first describe how each section of the stress response is analyzed, and then discuss the behavior as a function of cross-linker density.

The growth of the shear stress immediately after the initiation of strain is used to extract information about the elastic modulus of the interface (see insert in Fig. 2). In this regime of small strains, the linear response of the stress is assured so we may write [29]

$$\sigma(t) = \int_0^t dt' G(t-t') \gamma(t'), \quad (2)$$

where  $\gamma(t)$  is the strain history of the sample. We have implicitly assumed that there was no strain before  $t = 0$ . Expressing this in the frequency domain, prescribing the strain to be  $\gamma(t) = \dot{\gamma} t \theta(t)$  where  $\dot{\gamma}$  is a constant, and  $\theta(t)$  is the usual step function, and expanding for  $t \rightarrow 0^+$ , we may write

$$\sigma(t) = \dot{\gamma} \int_0^t dt' \int_{-\infty}^{\infty} \frac{d\omega}{2\pi} G^*(\omega) e^{i\omega(t-t')} t' = G'(0) \dot{\gamma} t + \mathcal{O}(t^2), \quad (3)$$

where  $G^*(\omega) = G'(\omega) + iG''(\omega)$  is the Fourier transform of the viscoelastic shear modulus appearing in Eq. 2. Thus, for an ideal apparatus, we may interpret the slope of the initial rise in the stress in terms of the integral of the time-dependent elastic modulus

$$G'(0) = \lim_{t \rightarrow 0^+} \int_{-\infty}^{\infty} G'(\omega) e^{-i\omega t} \frac{d\omega}{2\pi}. \quad (4)$$

If the storage modulus is dominated by its long-time or low-frequency behavior, we may interpret the initial growth in the stress in terms of the elastic modulus of F-actin network associated with the Langmuir monolayer.

It should be noted that because the inner cylinder moves during the initial shear, one has to define the initial strain carefully. Therefore, in practice, the elastic modulus  $G'(0) \equiv G$  is measured using the following relation [30]:

$$G = \frac{\omega}{\Omega - \omega} \left( \frac{\kappa}{4\pi} \right) \left( \frac{1}{r_i^2} - \frac{1}{r_o^2} \right), \quad (5)$$

where  $\Omega$  is the angular rotation speed of the outer cylinder,  $\omega$  is the angular rotation speed of the inner cylinder,  $\kappa$  is the torsion constant of the supporting wire, and  $r_o$

is the outer cylinder radius. For more details on the this procedure, see Ref. [30].

Because the initial stress rise is approximately linear and the subsequent decay is exponential, it is reasonable to treat the material as a simple Maxwell fluid having a single stress relaxation time  $\tau$ , so that we may write

$$G^*(\omega) = \frac{G_0}{1 - i\omega\tau} \quad (6)$$

for the viscoelastic shear modulus, then using Eqs. 3 and 4, this allows us to identify our previously defined elastic modulus (based on the initial slope of the stress versus time curve) with  $G_0$ .

The other element of the Maxwell model is the stress relaxation time  $\tau$ . At the cessation of shear (at time  $T$ ), the stress relaxes as

$$\sigma(t) = G_0 \dot{\gamma} \tau e^{-(t-T)/\tau}, \quad (7)$$

for times  $t > T$ . An example of the stress relaxation is given in Fig. 3. By considering the log of the stress versus time, we are able to extract a relaxation time. Over the range of cross-linker densities studied, we observed no dependence of the relaxation time on cross-linker density. The value is  $\tau = (1.58 \pm 0.3)$  s. Closer examination of the stress relaxation reveals that it is better fit by a double exponential decay and that there is no significant improvement in the fit resulting from the use of additional exponential decays. There is a short relaxation time on the order of seconds for which the exponential has an amplitude roughly an order of magnitude larger than the second exponential. For the example in Fig. 3, the short relaxation time is 1.9 s with amplitude  $32.1 \times 10^{-6}$  N/m and the long relaxation time is 15.4 s with an amplitude of  $1.8 \times 10^{-6}$  N/m.

In the context of the Maxwell model, the steady-state viscosity is given by  $\tau G_0$ . This provides a consistency check on the model. In our case, if we use the value of  $G_0$  from the flow start-up, the values of  $\tau G_0$  range between  $10^{-3}$  and  $10^{-4}$  Ns/m over the shear rates studied. In contrast, the viscosity ranged from  $10^{-4}$  and  $10^{-5}$  Ns/m, roughly a factor of 10 smaller. Surprisingly, both quantities had roughly the same scaling with shear-rate. Combining this particular break-down of the Maxwell model with the fact that the relaxation times  $\tau$  are independent of cross-linker density suggests the following picture for the system. During the initial applied strain, the system is sufficiently linear that we are able to measure the elastic response due to both stretching of cross-linked filaments and the interactions between domains of cross-linked filaments. Once the system flows, the dissipation is dominated by the interaction between the domains. Likewise, the dominant stress relaxation is between domains, and therefore,  $\tau$  does not depend on the cross-linking concentration. Finally, the break up of the domains during steady shear explains the inconsistency between  $G_0$  measured at start-up and from flow cessation. It is the role played by the cross-linkers that results in a larger

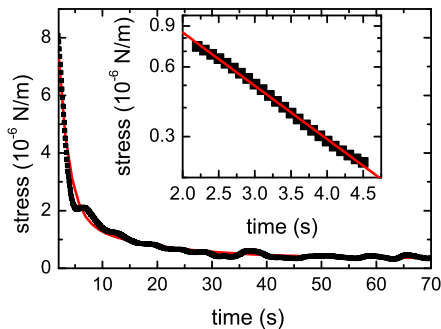


FIG. 3: (color online) The torque versus time for the cessation of flow for the F-actin network with a cross linker density of  $R = 0.005$ . The outer cylinder was rotated with a fixed angular velocity of 0.4 rad/sec (shear rate 1.3), and stopped at  $t = 0$  s. The solid line is a fit to a double exponential decay as discussed in the text. Insert: Semi-log plot of the same data showing just the early time behavior and the dominant relaxation time. The solid red line is a fit to the data with time constant 1.9 s.

measured elastic modulus than one would expect for the Maxwell model.

To further test this picture, we consider the steady-state viscosity in more detail. The steady-state viscosity of the system is strongly shear-rate dependent (see Fig. 4). This is another reason that we are not surprised at the failure of the Maxwell model to completely describe the behavior, as the Maxwell model is a linear response model. We return to this point in our conclusions – see section IV. It is worth pointing out another sign of the failure of linear rheological models; the observed stress overshoot at the initiation of flow – see Figs. 2. Stress overshoot has been observed in experiments [31, 32] and in molecular dynamics simulations [33] of flexible polymer melts and solutions although care must be taken to distinguish this effect from systematic errors introduced by the rheometer [34]. Theoretical models of this nonlinear rheological effect have been proposed for polymeric liquids [35, 36] and even metallic glasses [37].

Because our system operates in a wide-gap mode, we will review how the viscosity is measured. Before discussing the nonlinear response of our system, it is useful to recall the response of a Newtonian fluid in a Couette rheometer with an arbitrary gap width. Solving the Navier-Stokes equations for the Couette flow [38] in steady-state between a rotating outer cylinder of radius  $r_o$  at speed  $v_o = \Omega r_o$  and a stationary inner cylinder of radius  $r_i$  we find that the only non-vanishing component of the rate of strain tensor  $\dot{\gamma}_{r\theta} = \dot{\gamma}$  takes the form

$$\dot{\gamma} = \frac{2\Omega r_o^2}{r_o^2 - r_i^2} \left( \frac{r_i}{r} \right)^2. \quad (8)$$

For a viscous, Newtonian fluid with linear relationship between the shear stress and the shear rate of strain, we

find that the torque can be related to the viscosity and rotation rate of the outer cylinder by [28]

$$\mathcal{T} = \frac{4\pi\eta r_i^2 r_o^2 \Omega}{(r_o^2 - r_i^2)}, \quad (9)$$

where,  $\eta$  is the surface viscosity. The aqueous subphase makes only a small contribution to the shear stresses in the system, so using the above relation Eq. 9 we can determine the effective viscosity of the interface in steady-state. An important feature of this relationship is the fact that the torque is a linear function of the rotation rate. In Fig. 4(a) we show the dependence of the torque  $\mathcal{T}$  on the rotation rate of the outer cylinder in steady state for both the unlinked actin network and the highest  $R$  value used. The (red) line is the best linear fit to the data on this logarithmic plot demonstrating a power-law dependence of the measured torque upon the shear rate.

Systems displaying this sort of nonlinear rheology are typically referred to as power-law fluids [39, 40], in which case one has the following relation between stress and rate of strain:

$$\sigma = \eta' \dot{\gamma}^n \propto \Omega^n, \quad (10)$$

where  $n$  is the exponent of the power-law fluid. In such a system, the torque versus rotation curve can be used to determine the exponent in the relation between stress and rate of strain. In this case, the effective viscosity ( $\sigma/\dot{\gamma}$ ) depends on shear rate as

$$\eta = \eta' \dot{\gamma}^{n-1}, \quad (11)$$

As discussed above for a case of a Newtonian fluid, it is possible to extract the effective viscosity from the measured torque  $\mathcal{T}$  at the inner cylinder and the imposed angular velocity of the outer cylinder  $\Omega$ , once the value of  $n$  is determined. In this case, however, we must modify Eq. 9 to account for the nonlinear dependence of stress on shear rate given by Eq. 11.

For a power-law fluid, the relationship between the measured stress at the inner rotor (determined from Eq. 1) and the angular velocity of the outer rotor  $\Omega$  is given by [28]

$$\sigma = \eta \dot{\gamma} = \frac{2\eta}{n} \frac{\Omega r_o^{2/n}}{r_o^{2/n} - r_i^{2/n}}, \quad (12)$$

so the effective viscosity can be calculated in terms of the experimentally measured parameters using Eqs. 1 and 12. We find that

$$\eta = \frac{\mathcal{T}(r_o^{2/n} - r_i^{2/n})n}{4\pi\Omega r_i^2 r_o^{2/n}}. \quad (13)$$

One does have to be concerned that slip at the walls may play a role in influencing the measured values of the viscosity. Based on previous work, however, we do not expect slip to play a role in this system [41].

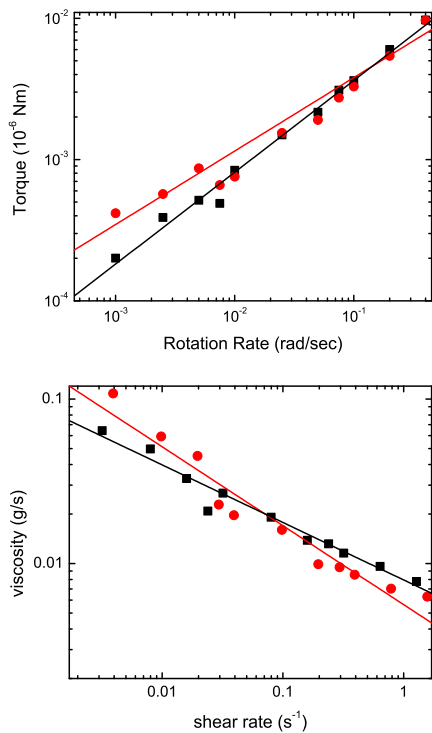


FIG. 4: (color online) Torque and surface viscosity measurements on an uncross-linked F-actin network ( $R = 0$ , black squares) and a F-actin network with  $R = 0.04$  cross linker density (red circles) at standard concentration associated with the lipid monolayer. (a) Torque vs. rotation rate of the outer cylinder showing a power-law dependence expected for a fluid in which the viscosity decays as a power of shear rate. See Eq. 11. The lines are the best fits to the data. (b) The surface viscosity of the composite monolayer extracted from the data in (a). See Eqs. 12,13. The lines are the best fit to the data.

In Fig. 4(b) we extract the effective viscosity from these data using Eq. 13. The (red) line again represents the best linear fit to the data on a logarithmic plot and shows that the effective viscosity indeed has a power-law dependence on shear rate. The F-actin network is shear thinning over all shear rates probed encompassing a range of  $10^{-2}\text{s}^{-1}$  to  $1\text{s}^{-1}$ . From these data we find that the exponent is best fit by  $n = 0.65 \pm 0.02$  for the uncross-linked sample and  $n = 0.52 \pm 0.04$  for the  $R = 0.04$ . Notice, the variation in the magnitude of the viscosity from increasing the cross linking density is small in the range of shear rates studied, but there appears to be a small, but measurable, impact on the exponent. This will be discussed in more detail later.

We vary the cross-linker concentration by increasing the fraction  $R$  of biotinylated to non-biotinylated G-actin in the polymerizing actin solution. As the concentration of cross-linkers increases, we observe well-defined trends in both the elastic response of the network at small strains and the steady-state, nonlinear rheology of the network. We expect that the actual number of

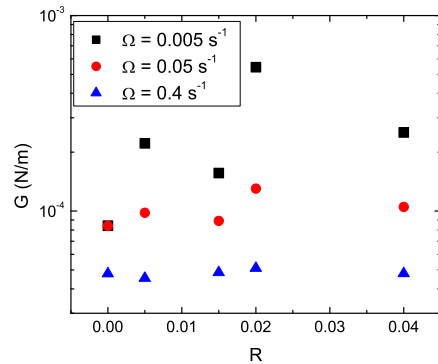


FIG. 5: (color online) The elastic modulus as extracted from the data shown in Fig. 2 vs. cross-linker density as measured by  $R$ .

cross-linkers in the network is proportional to the fraction of these cross-linking molecules. Clearly, at high enough concentrations of cross-linkers this linear relationship fails as steric constraints prevent the saturation of the available biotin-bonds.

In Fig. 5, we extract the elastic modulus Eq. 4 for a range of cross-linker concentrations. At each concentration we study the system at three different shear rates. At the lowest shear rate studied (black squares), we see a roughly linear increase in elastic modulus with cross-linker concentration. At the highest concentration for which we took data, the modulus appears to have reached a plateau, which we attribute to the failure of the system to make use of the additional cross-linkers on the filaments. The linear dependence of the elastic modulus is expected in an affinely deforming [17, 18] network, and the eventual saturation is not unexpected for reasons discussed above. Turning to the data taken at the intermediate shear rate (red circles) we see that the linear increase of the observed modulus with cross-linker concentration remains but at a smaller slope. Finally, at the highest shear rate used (blue triangles), there is no observable dependence of the modulus on the cross-linker concentration.

We also note from Fig. 5 that the magnitude of the elastic response of the network extracted from the initiation of shear decreases with increasing shear rate. This is illustrated in more detail in Fig. 6. The measured modulus decreases with imposed shear rate in a manner consistent with a power-law

$$G'(\dot{\gamma}) \sim \dot{\gamma}^{-0.4}. \quad (14)$$

The data corresponding to higher shear rates is essentially independent of cross-link density. We interpret this behavior as suggesting that the observed modulus in this high shear rate regime is due to the mechanics of interacting domains of cross-linked filaments, and not elastic strain energy stored in individual cross-linked networks.

For comparison, it is worth returning to the impact of the cross-link density on the viscosity. What is in-



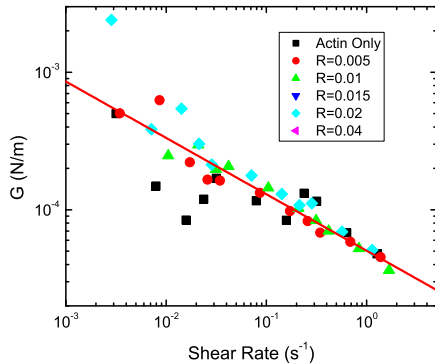


FIG. 6: (color online) The elastic modulus vs. rotation rate of the outer cylinder for a range of cross-linker concentrations. The linear dependence (best fit line in red) demonstrates a power law dependence of the elastic modulus on rotation rate with an exponent of  $-0.4$ .

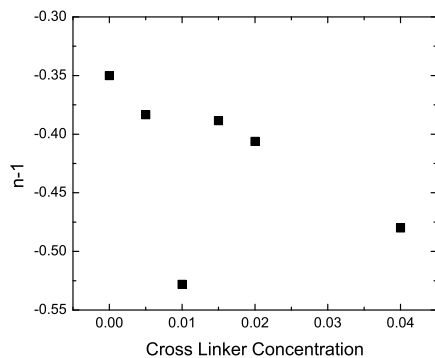


FIG. 7: The dependence of the best fit value of  $n - 1$  for the dependence of the viscosity on rate of strain as a function of ratio of cross-linker to G-actin concentration.

interesting is that even though the density of cross-linkers does not appear to impact the magnitude of the measured viscosity significantly, there is a weak effect on the exponent  $n$  from the fits to a power-law fluid. In Fig. 7, we plot the exponent for the viscosity ( $n - 1$ ), where  $n$  is defined in Eq. 11. As the cross-linker concentration increases the exponent  $n$  decreases from  $n = 0.65 \pm 0.02$  to  $n = 0.52 \pm 0.04$ . Unlike the case of the elastic modulus, we do not find any saturation of the exponent with increasing cross-linker concentration.

Sollich et al. have proposed [21] this sort of power-law rheology can arise rather generically in the mechanics of structurally disordered, soft materials that exhibit at large number of metastable microstates consistent with a given applied stress. Given our microscopic picture of long F-actin filaments slipping past each other under the applied shear stress, this unifying picture of the rheology of soft disordered materials appears applicable to the strongly sheared network. Due to steric interactions between the filaments we expect to find such rheological behavior in both the cross-linked (by biotin-streptavidin)

and the uncross-linked samples.

In the Sollich framework, we may interpret the power law exponent  $n$  in terms of as “effective noise temperature”  $x = n + 1$ . Over the range of cross-linker concentrations studied  $x$  ranges from  $x = 1.35$  to  $x = 1.45$ . Given these values of the effective noise temperature, the system is predicted to exhibit power-law rheology in steady-state. Moreover, they predict that the system should have a vanishing yield stress. In other words it should flow in the limit of vanishingly small applied shears. While we have focused on the steady-flow behavior of the F-actin/monolayer material, our preliminary low shear stress data are consistent with this prediction. We will report more fully on the extremely low shear regime elsewhere [19].

#### IV. CONCLUSIONS

The complex mechanics of cross-linked F-actin networks underlies the dynamics and mechanics of living cells. While there has been a great deal of recent work on development of *in vitro* semiflexible network models of the cytoskeleton and measurements of their rheology, comparatively little effort has been expended on the development of analogous model systems to explore the mechanical interaction of the cytoskeleton with the plasma membrane. Here we have reported on just such a simplified model system that incorporates a mechanical coupling between a cross-linked semiflexible network and a lipid monolayer that mimics one leaflet of the plasma membrane of the cell. This model is clearly a minimal description of cytoskeletal networks mechanically associated with a liquid lipid phase boundary.

We have characterized the steady-state shear response of this composite material and found it to be well-described at higher shear rates by a power-law fluid model with an exponent consistent with those obtained from the soft glassy rheology picture of soft disordered materials. Such a correspondence is not entirely unexpected since, at the filament densities studied we imagine the system to consist of cross-linked and sterically confined long filaments that have numerous mechanical equilibria associated with moving uncross-linked strands of the network around each other.

We find at higher shear rates the effective viscosity decreases as  $\eta \sim \dot{\gamma}^{-0.4}$  and the elastic modulus decreases as  $G' \sim \dot{\gamma}^{-0.4}$ . The impact of the cross-linker density is interesting. At lower shear rates the elastic modulus depends roughly linearly upon cross-linker concentration although at higher shear rates we find essentially no rheological effect of the cross-linker concentration – see Fig. 5. The relaxation time after the cessation of flow is independent of the cross-linker density. The magnitude of the viscosity does not depend in any systematic way on cross-linker density, but the power-law used to describe the steady state viscosity decreases as a function of cross-linker density as shown in Fig. 7. Combining these

facts strongly suggests the following picture. The system is composed of clusters of cross-linked filaments. Our results implies that the observed mechanics are due to cross-linked filaments for low shear rates and strains (the elastic modulus), but at higher shear rates and strains, we are probing the interaction between clusters of cross-linker filaments (viscosity measurements, relaxation time after shear, and high shear rate elastic modulus).

From this picture, one is not surprised that the soft-glassy rheology ideas are appropriate for describing the steady state behavior, as the clusters will act as the fundamental mechanical elements in the soft glassy system. However, the dependence on the power-law exponent on cross-linker density provides an interesting direction for further study. This suggests that the details of the cluster-cluster interaction depend on the intrinsic mechanical properties of the clusters themselves. These are clearly dependent on the cross-linker density, as indicated by the low shear rate measurements of the elastic modulus. This presents us with an interesting system to study the impact of local mechanical properties on the macroscopic properties. A detailed study of the internal cluster properties will be the subject of future work using microrheological probes of the network associated with the lipid monolayer.

Microrheological studies will also be important as future studies incorporate additional complexity of linkers. For example, when one considers linkers that modify the local structure of the network (e.g. cause bundling) it will be important to have simultaneous access to both the local elastic response of the network and the system-

size, macroscopic elasticity of the gel. Additionally, a combination of traditional (Couette) rheometry and microrheological studies will be helpful in accessing the rheological effect of labile, physiological cross-linkers. It is clear that the low-frequency linear response of a network cross-linked by such transient bonds will be dramatically different from our biotin/streptavidin cross-linked system. In our case the network is a soft solid, having a finite elastic response at zero frequency. The network with labile cross-linkers, however, must flow like a liquid on time scales longer than the typical binding lifetime of a transient cross-linker. To probe this low-frequency rheology, the traditional Couette cell approach can be used. The addition of microrheology allows one to explore the higher frequency response of the system that is inaccessible to the macroscopic measurements. These high frequency data should show that the presence of labile cross-linkers affect only the low frequency response of the system. The effect of labile cross-linkers on the non-linear rheology of the system at high strains also remains to be considered.

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- [1] P.A. Janmey, S. Hvidt, J. Lamb, T.P. Stossel *Nature* **345**, 89 (1990).
  - [2] F.C. MacKintosh and P.A. Janmey, *Curr. Opin. Sol. St. and Mat. Sci.* **2**, 350 (1997).
  - [3] M.L. Gardel, J.H. Shin, F.C. MacKintosh, L. Madadevan, P. Matsudaira, and D.A. Weitz, *Science* **304**, 1301 (2004).
  - [4] M.L. Gardel, F. Nakamura, J.H. Hartwig, J.C. Crocker, T.P. Stossel, and D. A. Weitz, *Proc. Natl. Acad. Sci. USA* **103**, 1762 (2006).
  - [5] N. Desprat, A. Richert, J. Simeon, A. Asnacios, *Biophys. J.* **88**, 2224 (2005).
  - [6] M.F. Coughlin, M. Puig-de-Morales, P. Bursac, M. Mellema, E. Millet, J.J. Fredberg, *Biophys. J.* **90**, 2199 (2006).
  - [7] K.M. Van Citters, B.D. Hoffman, G. Massiera, and J.C. Crocker, *Biophys. J.* **91**, 3946 (2006).
  - [8] X. Trepate, L.H. Deng, S.S. An, D. Navajas, D.J. Tschumperlin, W.T. Gerhoffer, J.P. Butler, and J.J. Fredberg, *Nature* **447**, 592 (2007).
  - [9] E. Helfer, S. Harlepp, L. Bourdieu, J. Robert, F.C. MacKintosh, and D. Chatenay, *Phys. Rev. Lett.* **87**, 088103 (2001).
  - [10] E. Helfer, S. Harlepp, L. Bourdieu, J. Robert, F.C. MacKintosh, and D. Chatenay, *Phys. Rev. Lett.* **85**, 457 (2000).
  - [11] E. Helfer, S. Harlepp, L. Bourdieu, J. Robert, F.C. MacKintosh, and D. Chatenay, *Phys. Rev. E* **63**, 021904 (2001).
  - [12] V.G. Brunton, I.R.J. MacPherson, M.C. Frame, *Biochim. et Biophys. Acta – Mol. Cell Res.* **1692**, 121 (2004).
  - [13] B.A. DiDonna and Alex J. Levine, *Phys. Rev. Lett.* **97**, 068104 (2006).
  - [14] B.A. DiDonna and Alex J. Levine, *Phys. Rev. E* **75**, 041909 (2007).
  - [15] C.P. Broederaz, C. Storm, and F.C. MacKintosh, *arXiv:0710.3951* (2007).
  - [16] F.C. MacKintosh, J. Käs, and P.A. Janmey, *Phys. Rev. Lett.* **75**, 4425 (1995).
  - [17] David A. Head, A.J. Levine, and F.C. MacKintosh, *Phys. Rev. Lett.* **91**, 108102 (2003).
  - [18] J. Wilhelm and E. Frey, *Phys. Rev. Lett.* **91**, 108103 (2003).
  - [19] R. Walder, A.J. Levine, and M. Dennin, unpublished (2007).
  - [20] R. Walder and M. Dennin, unpublished (2007).
  - [21] P. Sollich, F. Lequeux, P. Hébraud, and M.E. Cates, *Phys. Rev. Lett.* **78**, 2020 (1997).
  - [22] C.F. Schmidt, M. Bärmann, G. Isenberg, and E. Sackmann, *Macromol.* **22**, 3638 (1989).
  - [23] J. Wehland, M. Osborn, and K. Weber, *Proc. Natl. Acad. Sci. (USA)* **74**, 5613 (1977).



- [24] M.A. Dichtl and E. Sackmann, N. J. Phys. **1**, 1 (1999).
- [25] K.Sengupta, L. Limozin, M. Tristl, I. Haase, M. Fischer, E. Sackmann Langmuir **22**, 5776 (2006).
- [26] R.S. Ghaskadvi and Michael Dennin, Rev. Sci. Instr., **69**, 3568 (1998).
- [27] L.D. Landau and E.M. Lifshitz, *Theory of Elasticity* 3<sup>rd</sup> Edition (Pergamon Press, Oxford, 1986).
- [28] R.B. Bird, R.C. Armstrong, and O. Hassager, *Dynamics of Polymeric Liquids* (Wiley, New York, 1977).
- [29] M. Doi and S.F. Edwards, *Theory of Polymer Dynamics*, (Clarendon Press, Oxford, 1986).
- [30] M. Dennin, Phys. Rev. E **70**, 041406 (2004).
- [31] T. Raible, A. Demarmels, J. Meissner, Poly. Bull. **1**, 397 (1979).
- [32] K. Osaki, T. Inoue, and T. Isomura, J. Polym. Sci. Part B: Polym. Phys. **38**, 2043 (2000).
- [33] J. D. Moore, S.T. Cui, H.D. Cochran, P.T. Cummings, Phys. Rev. E **60**, 6956 (1999).
- [34] J. Meissner, J. Appl. Polym. Sci. **16**, 2877 (1972).
- [35] N. Murayama, Coll. & Poly. Sci. **258**, 1354 (1980).
- [36] D. Pearson, E. Herbolzheimer, N. Grizzuti, and G. Marrucci, J. Polym. Sci.: Part B: Poly. Phys. **29**, 1589 (1991).
- [37] H.-S. Chen, H. Kato, and A. Inoue, Jpn. J. Appl. Phys. **39**, 1808 (2000).
- [38] L.D. Landau and E.M. Lifshitz, *Fluid Mechanics* 2<sup>rd</sup> Edition (Elsevier, Amsterdam, 2004).
- [39] H.A. Barnes, J.F. Hutton, and K. Walters, *An Introduction to Rheology* (Elsevier, Amsterdam, 1989).
- [40] S.D. Holdsworth, Trans. Inst. Chem. Eng. **71**, 139 (1993).
- [41] M. Twardos and M.Dennin, Langmuir, **19**, 3542 (2003).
- [42] W.P. Cox, E.H. Merz J. Polym. Sci. **28**, 619 (1958).